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Thesis

The Determination of Quinidine in Serum

by

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I N D E X

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INTRODUCTION

Quinidine belongs to that class of compounds known as the cinchona alkaloids. It is the dextro-isomer of the more familiar quinine and enters into numerous chemical reactions, yielding the same end-products as quinine. It is because of this fact that some of the methods developed for the quantitative determination of quinine, mentioned in the historical review, could be adapted to the quantitative determination of quinidine.

Quinidine sulfate has had a stormy pharmacopoeial history. Not only was it condemned by the Council on Pharmacy and Chemistry of the American Medical Association, but it was expelled vigorously from U. S. P. VIII and omitted from U. S. P. IX. Later it was found that quinidine sulfate could be used to re-establish the normal rhythm of the heart in auricullar fibrillation, a condition in which the walls of the auricles do not contract as a whole or in an effective manner. Thus the blood flow from the auricles to the ventricles is not aided by auricular systole. With such a discovery, all was forgiven and quinidine sulfate was restored to its rightful position in the U. S. P. X and is comfortably established in U. S. P. XI.

The purpose of this research is to establish a method for the quantitative determination of quinidine that will be

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suitable for clinical purposes. Such a method should of necessity entail brevity, simplicity and accuracy.

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For purely analytical purposes, the various chemical methods for the determination of alkaloids in body fluids may be classified into 5 major groups: namely the colorimetric, fluorometric, nephelometric, gravimetric, and volumetric. It is evident that each group has its own particular advantages and disadvantages, which are more inherent in the methods themselves than in the techniques involved. In this portion of the thesis the various methods, proceeding from the most useful and desirable to the least, will be discussed.

1. Colorimetric Methods:

Some may doubt the validity of placing the colorimetric methods before the fluorometric but I believe that while the fluorometric methods are more sensitive, the simple apparatus and technique involved in colorometric methods more than offsets their comparatively lower sensitivity.

As the name implies the colorimetric methods that have been developed for the alkaloids are based on their common property of forming colored complexes with various reagents and, as must be the case for any reliable method, the intensity of the color developed must vary directly with the amount of alkaloid present.

As early as 1927, Shevdskii[47] had developed an exceptionally sensitive test that was capable of measuring quinine even in

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concentrations of 1:200,000. He employed ether extracts of blood and developed a color suitable for measurement by the use of Mayer's reagent, $KI-HgI_2$. Unfortunately, as was pointed out by Smorodintzen and Adova[48] later, higher results are obtained then can be expected from the alkaloid itself. Troublesome precipitates are common and on the whole the method is lengthy and complicated. Shvedskii's method is limited to pure aqueous solutions of quinine. Rojahn[38] attempted to employ the method in order to determine strychnine, quinine, emetine and cinchonine in blood and he found that it was essentially accurate for only strychnine and emetine. Attempting to modify the procedure, Rojahn introduced picric acid to develop the color and could successfully determine all four of the alkaloids mentioned.

Vedder and Masen[55] have devised a colorimetric test for alkaloids in blood in which the alkaloid, in this case quinine, is extracted from the blood with ether. "The ether is evaporated, 5 cc of 2N sulfuric acid saturated with zinc sulfate is added, and the tube immersed in the boiling brine bath for 3 minutes in order to bring the quinine into solution. The solution while hot, is filtered through a no. 42 Whatman filter, and, after having cooled to room temperature, an aliquot of the filtrate, 3 cc is measured into a small test tube. Into 3

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similar test tubes measure 5 cc of each of 3 quinine standard solutions. Immerse both standards and unknown in a cold water bath for 5 minutes in order to bring them to the same temperature. Then add to the standard .1 cc and the unknown .06 cc of the gum arabic solution, followed by the same amounts of potassium bismuthous iodide reagent (0.1 cc to the standard and 0.06 cc to the unknown. Mix and compare immediately in the colorimeter with the standard set at 10 millimeters."

Vedder and Masen were completely aware of the errors involved in such a procedure and were completely emphatic in pointing out that the reaction is first of all non-specific. The bismuth iodine reagent will react with other alkaloids besides quinine or quinidine and in this respect will react even with other basic substances. Furthermore, the color developed is not stable and the method is not applicable when the alkaloid is below 2 mgm per liter.

In recent years the trend has been towards the development of colorimetric tests in which the alkaloids are coupled with acid dyes and in this, Prudhomme[36] must be considered as one of the most foremost pioneers. Prudhomme elaborated a simple colorimetric test for the determination of quinine in urine by adding an acid dye to urine and extracting with chloroform. The resulting color is compared to standards. Aside from this

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colorimetric test for quinine he has developed a general test for alkaloids in blood. A filtrate obtained by treating blood with sodium sulfate and normal sulfuric acid is buffered at pH 7 and a 2% solution of eosin is added. The red color developed is extracted with chloroform and compared to standards. Again, as is the common case, we find this method is limited in application because of the faint intensity of the colored complexes and more so because of the large adsorption of the colored complex on glasssurfaces.

Although Vedder and Masen were pioneers in the field of colorimetric tests for alkaloids it remained for Brodie and Undenfriend[3,4] to develop general procedures whereby many synthetic basic organic compounds and a number of alkaloids could be determined colorimetrically. " The principle of the method is that many organic bases combine with certain sulfonic acids to form molecular complexes which are highly soluble in organic solvents, and that the concentration of base in the organic solvent may be determined indirectly through a measurement of the concentration of the sulfonic acid in the organic reagent. The technique used to appraised the specificity of the procedure involves a comparison of the solubility characteristics of the pure compound with those of the substance or substances isolated from the biological

material and measured in the analytical procedure."

At this point I would like to Justify the time and space devoted to Brodie's work. While his work on the colorimetric determination of alkaloids is not too detailed, it has served as the stepping stone for my work and many other individual tests and as such merits close consideration.

Brodie describes and I quote verbatim a procedure for determining cinchonidine in plasma. This particular procedure has been subsequently modified and adapted by Morton K. Schwartz, working in conjuction with Dr. Norwood K. Schaffer and Dr. Burnham S. Walker, for the determination of quinidine in plasma.

"Procedure for plasma--Add 1 to 5 ml of Biological Sample and 1 ml 1N sodium hydroxide to 20 ml of ethylene dichloride in a 60 ml glass-stoppered bottle and shake for 5 minutes, preferably on a shaking apparatus. Decant the contents of the bottle into a 40 ml round bottomed tube and centrifuge for 10 minutes at 2500 R.P.M. to break the emulsion. Remove the supernatant aqueous layer by aspiration. Return the ethylene dichloride solution to a 60 ml glass-stoppered bottle (the original thoroughly rinsed out bottle may be used), restraining any coagulum present with a stirring rod. Add an equal volume of the alcoholic KOH solution and shake for 10 minutes.

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Transfer the contents to a 40 ml tube (the original rinsed out tube may be used) and centrifuge for 1 minute at 2500 R.P.M. Remove the supernatant aqueous layer completely by aspiration and decant the ethylene dichloride into a 60 ml glass-stoppered bottle (the original thoroughly rinsed out bottle may be used). Add 0.5 ml of methyl orange reagent and shake for 5 minutes. Decant into a 25 ml test tube and centrifuge for 5 minutes at 3000 R.P.M. Carefully remove all the supernatant layer by aspiration, decant the ethylene dichloride phases into a 25 ml test tube and recentrifuge for 5 minutes. Pipette 10 ml of the ethylene dichloride into a colorimeter tube containing 1 ml of the alcoholic sulfuric acid and mix thoroughly. Read in the colorimeter with a filter having a maximal transmission at 540 mu.

A reagent blank in which water is substituted for plasma is run through the same procedure and is used for setting the instrument to 100% transmission. This reagent blank should not give a transmission of less than 97 when ethylene dichloride plus the alcoholic sulfuric is used to set the instrument to 100 (Evelyn photoelectric colorimeter)."

Although Brodie has worked out general procedures for the cinchona alkaloids it remains for any interested person to modify and adapt them to any particular alkaloid. As has been

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stated Schwartz has recently applied Brodie's method for determining cinchonidine to the determination of quinidine in plasma and the method is quite successful since it is simple, quick and sufficiently sensitive.

The procedure as described above is inadequate for quinidine and it remained for Schwartz to make the necessary adjustments. For example, although Brodie recognizes the necessity of eliminating the alkali before the methyl orange is added, he proposes no way of doing it. The few drops that remain after aspiration can be, as Schwartz found, removed by washing with 1 ml of distilled water. It is interesting in this connection that the amount of wash water did not effect the results. From this the solubility of quinidine in ethylene dichloride as compared to water may be readily imagined.

Brodie advocates the use of a fresh reagent blank to set the Evelyn photoelectric colorimeter to 100 % transmission but in this respect Schwartz found that a stock solution could be prepared that was sufficiently stable when kept cold so as to eliminate the necessity of running concurrent blanks. It was finally decided that a mixture of ethylene dichloride and alcoholic sulfuric acid could be used to set the photometer to one hundred per cent transmission. The main objection to

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the ethylene dichloride method seems to be the high plasma blanks and the emulsions that result when the quinidine solution, the sodium hydroxide and the ethylene dichloride are shaken. The emulsion is of a troublesome mature since it is a semi-solid one and since it extends down into the bottom layer of ethylene chloride. Naturally attempts were made to overcome these obstacles. The emulsion is broken sufficiently by the addition of 1 ml of water to enable one to draw an aliquot of the ethylene dichloride by inserting a pipette into the tube. Again an emulsion may result when the aliquot is shaken with potassium hydroxide which necessitates a repetition of the above technique. Various devices were employed to eliminate the high plasma blanks none of which were successful. Trichloroacetic acid, tungstic acid, hydrochloric-heat and meta-phosphoric acid filtrates were prepared but to no avail. It was due largely to the objections raised in this last paragraph that I sought to adapt Brodie's work to a colorimetric test for quinidine that would embody all the fine points of the of the ethylene dichloride method and exclude some of its bad features.

2. Fluorometric Methods:

These methods are dependent on the fact that molecules or atoms, which have been excited by the absorption of light,

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emit light of a wavelength different from that of the incident radiation. Generally, the emitted light has a wavelength greater than that of the incident radiation. When the incident radiation is removed, the phenomena of fluorescence ceases. Usually, after the absorption of light the excited electrons give up their additional energy to neighboring atoms to be dissipated as heat. The atoms, alternatively, may reradiate part of its energy and fall back to the original state. If the return is made immediately the phenomena is called fluorescence, but if it is delayed it is called phosphorescence. Included among the many substances that fluoresce are petroleum, eosin, fluorite, certain metallic vapors and quinidine sulfate.

This characteristic fluorescence of quinidine sulfate when exposed to ultraviolet light, affords a method of quantitative analysis. As would be expected, the number of methods devised for the determination of quinidine on such a principle are limited.

Mention has already been made of a method employing both alkalimetry and fluorometry. Efimenko[9,10] exposes a chloroform-ethyl ether extract of blood containing quinidine to a quartz lamp and titrates the solution with bromine to the extinction of the fluorescence. Commonly iodine is employed, but Efimenko found that the results are better with bromine since the bromides

of the alkaloids are soluble while the iodides are not.

The number of methods employing fluorometric procedures are about equally divided into two general types. The fluorescence is either extinguished by titration or else measured in a fluorometer. Instances of the latter are the methods developed by kelsey and Geiling[25], Brodie and Udenfriend[3] and Unti[51].

The method of Kelsey and Geiling is exceptionally simple. Sodium hydroxide is added to blood and the mixture heated on a steam bath. The extraction of the quinidine is accomplished by ether. The ether is acidified with sulfuric acid and the intensity of the fluorescence measured in a fluorometer.

Brodie and Undenfriend dilute plasma with dilute phosphoric acid. The mixture is centrifuged and the fluorescence of the clear solution under ultraviolet light is measured in a photofluorometer.

The method developed by Unti is not sufficiently more accurate than the methods described and entails much more work. 1 ml of blood is dehydrated with sodium sulfate and then made alkaline with ammonium hydroxide. The extraction of the quinidine is made with an ether-chloroform mixture and sulfuric acid added. The fluorescence is compared to standard solutions prepared by dissolving quinidine in sulfuric acid.

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3. Volumetric Methods:

By far, the greatest number of methods developed for the determination of the alkaloids, particularly for quinine and quinidine, fall in this class. Most of the individual tests developed are outcrops of methods previously developed and only a few represent entirely original methods. As is usually the case, modifications are introduced until the methods bear but little resemblance to the original.

Up to 1926, the chief mode of determining the alkaloids was by volumetric analysis. Methods were developed for the alkaloids but on the whole the dissociation constants of the alkaloids and the titration curves of the latter were essentially ignored. It remained for Morton[32] to determine the dissociation constants of the alkaloids and gather information as to their general properties. He investigated the titration curves of quinine and quinidine being particularly interested in the pH prevailing at the equivalence and at the half-equivalence point. The titration curves for quinine and quinidine show little inflection at the half-way point and a more marked inflection at the equivalence point, when they are titrated with HCL. If one selects the first inflection point then a limited number of indicators is available. Bromocresol purple is available but this requires the use of a color standard containing a buffer solution in order to insure

stability of the color. If the equivalence point is chosen, the cinchona alkaloids may be accurately titrated without the use of a buffer solution by means of bromophenol blue. Morton summarizes the optimum condition for the titration of the cinchona alkaloids. The data accumulated by Morton is constantly referred to in the literature and has served to guide many of his successors.

Aside from the common volumetric methods, a great number of procedures are listed that are of the potentiometric variety. In such methods, a reagent is added gradually until the voltage changes very rapidly on the addition of a slight amount of reagent. This great change in voltage, comparatively speaking, is of sufficient magnitude to be read directly from a galvanometer but usually a potentiometer is employed. The voltages as noted on the potentiometer are plotted against the volume of the reagent added and the steepest part of such a plot is located by simple inspection. This represents the end point of the titration. As is often the case, when the end point is not sufficiently sharp, a plot of the change in voltage divided by the change in volume against the volume of the reagent added is made. On such a plot, the end point of the titration is represented by a sharp peak. The apparatus required is of the simple type, a voltmeter-potentiometer as

found in most well-equipped laboratories is entirely satisfactory.

Many methods applying the above principles to the determination of quinine and quinidine have been cited in the literature. Holt and Kahlenburg[22] investigated the possibilities of various electrodes. Ag-W, Sb-C and W-C couples were tried as electrodes. The Ag-W was adjudged the most satisfactory. It is interesting that the potentiometric titrations of the alkaloids were entirely satisfactory by this method with the exceptions of quinine and quinidine. The results obtained from the potentiometric titrations were in good agreement with the results obtained with methyl red as an indicator and with the expected calculated results. The choice of methyl red as an indicator was fortunate since Eiderman has shown that the end point is exceptionally sharp and should be preferred to methyl orange that is commonly used.

Prideaux and Winfield[35] employed a quinhydrone electrode in their potentiometric measurements and were successful in titrating 0.10-0.05 molar quinine solutions. In connection with the latter, they cite experimental evidence to justify the use of p-nitrophenol and bromocresol purple as indicator for 0.10-.05 molar quinine solutions. The latter two indicators are used extensively in analysis for quinine.

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Schemmer and Hoch[41] attempted to push volumetric analysis still further and accordingly investigated the possibilities of employing displacement titrations, potentiometric titrations and acetone titrations of alkaloid salts. The findings of such an investigation were extremely favorable when applied to pure aqueous solutions, but in the presence of proteins and degradations products of such the results were poor. Evidently, such methods cannot be applied to blood.

Thomis[49], taking the cue from methods of Schlemmer and Koch has devised a general volumetric method for determining alkaloids that deviates only ± .1% from actual theory. A suitable indicator is determined experimentally for each alkaloid and painstaking devices are used to minimize errors. A thermomicroburet is used by Thomis which not only gives the exact temperature but permits one to make temperature corrections of the volumes used.

Various indicators have been successfully employed in the volumetric procedures for alkaloids, depending on the particular properties of the alkaloids under question. Malachite green has been used extensively as an indicator in the titration of alkaloids with silicoduodecitungstic acid but the end point is not sufficiently sharp. Feinstein[15] has substituted night blue for malachite green and this has been

found to be decidely more satisfactory. In the course of the titration of the alkaloid, a colloidal suspension is formed. When about 90% of the alkaloid has combined, the precipitate becomes flocculent. A portion of the supernatant liquid is transferred to a spot plate and a drop of night blue indicator solution added. As long as the alkaloid is in excess, the color on the spot plate is brown. When an excess of the reagent is added, it changes to blue. The main objection is that the night blue has to be made fresh each day. Schulek[43] employs a most unusual indicator. The quinidnie ~~is~~ to be determined is placed in some HCL and KBR is added. The solution is them titrated with potassium bromate, using alcoholic ethoxychyrsoidine as the indicator.

Papavassiliou[34] has developed one of the simplest methods that utilizes reagents commonly employed in volumetric procedures. An excess of potassium permanganate is added to an acid solution of the alkaloid. A definite weight of oxalic acid, in slight excess, is introduced and the excess is then back titrated with potassium permanganate.

The methods cited above, while tedious, can certainly be utilized by most clinical labatories. In contrast to these, other volumetric methods have been devised that certainly have limited clinical application. Grant,[19] for instance, has

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devised a very accurate determination for quinidine combing fluorometry with alkalimetry. An acidified quinidine solution is titrated with standard sodium hydroxide and the end point is evidenced by the disappearance of the fluorescence due to quinidine. The titration is carried out in a dark room under a verical beam of ultraviolet light. The author claims that the change is striking and that accurate titrations can be made of quinidine solutions which are .0001 N or weaker. Such a method would suffice for clinical purposes only in the absence of other methods.

4. Nephometric Methods

Most commonly, the comparison of two solutions is based on their respective colors. This by no means exhausts the possibilities though, since two solutions may be compared by virtue of the fact that each contains a dilute suspension of colored material instead of a true colored solution. Naturally, the latter is only true if the suspension does not flocculate or settle out before the comparison is made. Thus, a common method for the analysis of silver involves the comparison of silver chloride.

Similarly, the turbidities of two solutions may be compared since part of the light that passes through the solutions is absorbed even through the solution is not colored. The

nephelometer is based on such a priniciple. A beam of light strikes the tube containing the solution at right angles to the axis of the tube and the scattered light is measured.

Most of the nephelometric methods developed for the alkaloids involve the addition of silicotungstic acid to the alkaloid and then a comparisonof the resulting turbidity made with standards. Unfortunately, as Kyker and Lewis[27] discovered, the turbidity resulting from the addition of silicotungstic acid to quinidine settles out too rapidly to be measured. Nephelometric methods using silicotungstic acid for quinine, on the other hand, are extremely sensitive and the turbidity does not settle out so rapidly.

Stirken and Helrgat introduced an entirely new reagent to develop a turbidity with the alkaloids. They employ a mixture of sodium arsenate and ammonium molybdate and claim that it is the most sensitive reagent known, giving permanent opalescence even with dilutions of 1:2,000,000. In addition, the reagent is stable for 5 or 6 months and gives no opalescence with blood free from quinine or quinidine.

5. Gravimetric Methods:

Clinical methods for the alkaloids, employing gravimetric procedures, are relatively few in number. They are, as a rule, laborious and can detect only large amounts when microtechnique is not employed.

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Monet[31] has developed both gravimetric and volumetric procedures for alkaloids. In his gravimetric procedure, the alkaloids are precipitated with HCNS and the salts weighed. Sticht, on the other hand, precipitates quinine as the herapathite. An alcholic extract of blood is acidified with sulfuric acid and an iodine solution added. The resulting solution is filtered and the precipitate washed with alcohol saturated with herapathite. The precipitate is dried and weighed.

Miscellaneous Methods:

In addition to those methods described, we find a few miscellaneous ones. Lapp, in 1937, took advantage of the optical rotating power of quinine and developed a procedure for its quanitative determination employing a polarimeter.

The absorption spectra of the various alkaloids have been inveestigated in the hopes of devising new spectographic tests for the alkaloids, but the spectra are able to distinguish between the alkaloids only qualitatively.

EXPERIMENTAL PROCEDURE

BENZENE as the SOLVENT

After a review of the pertinent literature for the purpose of devising a colorimetric test for quinidine that would be suitable for clinical purposes, it remained only to select some promising and already fairly well developed procedure and adant it to my needs. Of all the methods examined, Brodie's test employing benzene as the extracting medium seemed to be one which was flexible enough to lend itself to my purposes. Accordingly I investigated the possibilities. In order to facilitate the dissussion and conclusions drawn from my experimental work, Brodie's method as such should be quoted verbatim for reference purposes at this point.

"Procedure--Add to 1 to 10 ml of biological sample (containing up to 5 gamma of cinchonine) and 1 ml of 2.5N NaOH to 30 ml of benzene in a 60 ml glass-stoppered bottle and shake for ten minutes on a shaking apparatus. Allow the phases to separate, centrifuging the bottle if necessary. Add 0.5 ml of isoamyl alcohol and mix with the benzene phase so as not to disturb the aqueous phase. Transfer as much of the benzene phase as possible to a glass-stoppered centrifuge tube containing 0.5ml of 1 N HCL. Shake for 5 minutes and then centrifuge.

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"Carefully remove the benzene phase by aspiration. Transfer at least 0.3 ml of the aqueous to a micro colorimeter tube and determine the optical density of the methyl orange solution at a wave length of 515 mu, using the Coleman model 6 spectrophotometer adapted to micro spectrophotometry as described in the procedure for pamaquine.

A reagent blank in which water is substituted for plasma is run through the above procedure and is used for setting the instrument to zero optical density. The reagent blank should not give an optical density of more than 0.010 when 1 N HCL is used to set the instrument at zero optical density."

Thus, this method is essentially one in which a sulfonic acid, methyl orange, is coupled with an alkaloid to form complexes of high molecular weight. The intensity of the complex is measured in the Coleman this And serves as an index to the amount of alkaloid present, since the amount of sulfonic acid entering the reaction is directly proportional to the amount of alkaloid already contained in the benzene phase. The sulfonic acid chosen by Brodie is methyl orange because of its high color index and since the methyl orange-alkaloid complex is highly soluble in benzene.

EXPERIMENTAL PROCEDURE

REMARKS on the SOLVENT

"Carefully remove the benzene phase by aspiration.

Transfer at least 0.3 ml of the aqueous to a micro colorimeter tube and determine the optical density of the methyl orange solution at a wave length of 515 mμ, using the Coleman model C spectrophotometer adapted to micro spectrophotometry as described in the procedure for pamaquine.

A reagent blank in which water is substituted for plasma is run through the above procedure and is used for setting the instrument to zero optical density. The reagent blank should not give an optical density of more than 0.010 when 1 N HCl is used to set the instrument at zero optical density."

Thus, this method is essentially one in which a sulfonic acid, methyl orange, is coupled with an alkaloid to form complexes of high molecular weight. The intensity of the complex is measured in the Coleman this serves as an index to the amount of alkaloid present, since the amount of sulfonic acid entering the reaction is directly proportional to the amount of alkaloid already contained in the benzene phase. The sulfonic acid chosen by Brodie is methyl orange because of its high color index and since the methyl orange-alkaloid complex is highly soluble in benzene.

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Reagents

1. Quinidine Standard: A stock solution is prepared by dissolving 120.7 mgs of quinidine sulfate in one liter of .1 N sulfuric acid. Aliquots of this solution are used and diluted with distilled water to give the test solutions of any particular strength.

2. 10% sodium hydroxide

3. 1 N hydrocloric acid

4. .5% boric acid

5. Benzene: This is purified by successive washings with one-fifth its volume of 1N Sodium hydroxide, 1 N hydrochloric acid and finally with distilled water.

6. Isoamyl alcohol: this is washed first with one-fifth its volume of 1 N hydrochloric acid followed by successive washings with distilled water.

7. Methyl orange solution: a solution (saturated) of methyl orange is prepared by dissolving the sodium salt of methyl orange in 0.5 M boric acid by gentle heating and allowing the solution to cool to room temperature. The excess methyl orange is filtered off. The boric acid serves to buffer the pH at 5 which is optimum for complex formation.

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APPARATUS

A shaking machine, 50 ml glass-stoppered graduate cylinder, 100 ml glass-stoppered volumetric flasks, standard volumetric and graduated pipettes, 50 ml centrifuge tubes, 40 ml test tubes, a centrifuge capable of attaining a speed of 2500 R.P.M., and a Coleman Photoeletric Meter.

DISCUSSION OF EXPERIMENTAL PROCEDURE

The procedure as described by Brodie was actually used per se and a few runs of 7.5 and 10 micrograms per 5 ml of distilled water were tried. 30.0 ml of benzene were used as the extracting medium and a 20 ml aliquot of the benzene solution containing the methyl orange- quinidine complexes was aspirated from the centrifuge tube and added to 2 ml$ of 1 N HCL in a 100 ml volumetric flask. THe results obtained were erratic . Such difficulties were encountered by Schwartz in the ethylene chloride method but he was able to eliminate them by washing the benzene phase with 1 ml of distilled water. The optimum pH, as stated previously, for complex formation is 5, so great care must be taken to remove the alkali before the methyl orange is added. Although Schwartz was able to do so. This is probably due to the more advantageous distribution of quinidine between ethylene cloride and water

EXPERIMENTAL PROCEDURE

BENZENE as the SOLVENT

APPARATUS

A shaking machine, 50 ml glass-stoppered graduate cylinder, 100 ml glass-stoppered volumetric flasks, standard volumetric and greased pipettes, 50 ml centrifuge tubes, 40 ml test tubes, a centrifuge capable of attaining a speed of 2800 R.P.M., and a Coleman Photoelectric Meter.

DISCUSSION OF EXPERIMENTAL PROCEDURE

The procedure as described by Brodie was actually used per se and a few runs of 7.5 and 10 micrograms per 5 ml of distilled water were tried. 30.0 ml of benzene were used as the extracting medium and a 20 ml aliquot of the benzene solution containing the methyl orange- quinidine complexes was aspirated from the centrifuge tube and added to 2 ml of 1 N HCl in a 100 ml volumetric flask. The results obtained were erratic. Such difficulties were encountered by Sobwarts in the ethylene chloride method but he was able to eliminate them by washing the benzene phase with 1 ml of distilled water. The optimum pH, as stated previously, for complex formation is 5, so great care must be taken to remove the alkali before the methyl orange is added. Although Sobwarts was able to do so. This is probably due to the more advantageous distribution of quinidine between ethylene chloride and water

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as compared to its distribution between benzene and water.

It is hard to understand why only a 20 ml aliquot was advocated, since such a process will result in only a two-thirds recovery of the total colored complex thus lowering the sensitivity of the method appreciably. Realizing this I decanted all the benzene layer containing the colored complex into the flask containing the 1 N HCL but again the results were not consistent and reproducible. Evidently some of the excess methyl was decanted into the acid solution along with the benzene layer and this gives the same pink color as the colored complex when acidified. It was evident from the data thus far that the distribution of quinidine between the benzene and water is not too favorable; this was out born by attempting a few double extractions. The amount of color which resulted from the second extraction was quite appreciable. In order to promote a more favorable distribution the volume of benzene was increased to 47 ml. This particular volume was decided upon since it would serve to extract about 8 per cent more quinidine and still keep within the limits of the 50 ml glass-ware especially the centrifuge tubes

Because of the negligible polarity of benzene, adsorption of quinidine on glass surfaces is extremely large. The

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BENZENE as the SOLVENT

necessity of adding some highly polar substance like isoamyl alcohol to prevent this is of paramount importance. This was vividly demonstrated. The procedure as outlined by Brodie was followed first by adding the alcohol and second by omitting the alcohol. In both cases the concentration to be determined was 10 micrograms. In the first instance, the results were consistent and gave an average reading on the Coleman of 68 versus 91 for the determination when the isoamyl was omitted.

To assure an excess of methyl orange when determinations for high concentrations were attempted it seemed advisable to use a concentrated solution of methyl orange instead of the solution proposed by Brodie. A most successful modification introduced in the benzene method was a way of removing the benzene from the initial aqueous layer, and the benzene containing the methyl orange-quinidine complex from the excess of methyl orange. It was found that an excellent separation could be made by the use of a capillary aspirator connected to the tap water aspirator. The layer to be removed can be caught easily in a test tube and the separation made to within 0.1 ml. Using such a device a 43 ml aliquot was taken instead of the 20 that Brodie suggested. Thus the recovery of the colored complex was increased from 67% to 92%. Combining the individual

EXPERIMENTAL PROCEDURE

BENZENE as the SOLVENT

EXPERIMENTAL PROCEDURE

BENZENE as the SOLVENT

modifications a standard curve for quinidine was determined. The procedure finally adopted being as follows:

Add 1 ml of quinidine standard solution, 4 ml of H_2O and 1.0 ml of 10 per cent sodium hydroxide to 47 ml of benzene in a 50 ml glass-stoppered graduate cylinder and shake for 15 minutes on the shaker. Allow the phases to separate. Add 0.5 ml of isoamyl alcohol and mix with the benzene phase without disturbing the aqueous phase. Aspirate the benzene layer into a test tube and pour all of this into a 100 ml volumetric flask containing 0.5 ml of the saturated methyl orange solution. Shake for one-half hour on the shaker. Centrifuge the solution for 10 minutes at 2500 R.P.M. Aspirate the benzene layer again and pour 43 ml of it into a 160 ml volumetric flask containing 2 ml of 1N HCL. Shake for 20 minutes. Centrifuge the solution for 10 minutes at 2500 R.P.M. Insert a 2 ml pipette into the acid layer after aspirating off the benzene layer. Transfer 1.5 ml of this to a microcoleman tube and read the optical density of the solution, setting the instrument to zero absorbance with 1.5 ml of 1N HCL at a wave length of 515 m .

The relationship between L and the concentration is not

EXPERIMENTAL PROCEDURE

BENZENE as the SOLVENT

linear under the cited experimental conditions. Nevertheless, the results are consistent and reproducible so they may be used. That the colored complex does follow Beer's law was quickly ascertained by diluting the color developed from a 40 microgram sample twofold with 1N HCL and this in turn twofold etc. The intensity of each of the resulting colors was measured and a plot of concentration versus L made which turned out to be definitely linear. The latter shows beyond doubt that the non-linearity of the experimentally determined curve is not inherent in the colored complex itself but rather that it is a consequence of the solvent and the experimental conditions.

EXPERIMENTAL PROCEDURE

BENZENE as the SOLVENT

EXPERIMENTAL DATA

BENZENE as the EXTRACTING MEDIUM

Quinidine Standard Curve:

47 ml of Benzene

Micrograms Quinidine/5 ml	G(% Transmittance)	L(2-Log G)
0.0	98	.0088
	98	.0088
5.0	84^{3}	.0718
	84	.0757
7.5	75_{3}	.1264
	76^{3}	.1149
	79^{2}	.0982
10.0	64^{3}	.1888
	64_{3}	.1888
	65^{2}	.1838
20.0	56^{2}	.2480
	56_{1}	.2518
	55^{1}	.2596
30.0	51	.2924
	51	.2924
	52	.2596
40.0	47^{1}	.325
	50	.308
	47	.328

EXPERIMENTAL DATA

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0.0	98	.0088
	98	.0088
5.0	84	.0738
	84	.0757
7.5	76	.1264
	76	.1149
	72	.0982
10.0	64	.1888
	64	.1888
	65	.1838
20.0	56	.2480
	56	.2518
	55	.2590
30.0	51	.2924
	51	.2924
	52	.2860
40.0	47	.328
	50	.308
	47	.328

EXPERIMENTAL DATA

BENZENE as the EXTRACTING MEDIUM

Quinidine Standard Curve:

30 ml of Benzene

Micrograms Quinidine/5 ml	G(% Transmittance)	L(2-Log G)
7.5	80	.0969
	74	.I308
	73	.I337
	70	.I549
I0.0	76	.II92
	74	.I308
	75	.I278
	77	.II2I
	80	.0969

Double Extraction

Micrograms Quinidine/5 ml	G(% Transmittance)	L(2-Log G)
I0.0	72[1]	.I4I2
	64[2]	.I888

Wavelength of Maximum Absorption

Wavelength (mμ)	G(% Transmittance)
515	52
	53
520	53
	53[1]

EXPERIMENTAL DATA

BENZENE as the EXTRACTING MEDIUM

Wavelength of Maxium Absorption

Wavelength (mμ)	G(% Transmittance)
525	54[1] 54
530	56 55[3]
535	58 58
540	60[2] 61
545	64[3] 64
550	68 67[3]
555	71[1] 711
560	75 75
565	79 79
570	82[2] 82[2]
575	86[1] 86[1]
580	88[2] 88[2]
585	90[1] 91

EXPERIMENTAL DATA

BENZENE as the EXTRACTING MEDIUM

Wavelength of Maxium Absorption

Wavelength (mμ)	G(% Transmittance)
490	57^{3} 57^{2}
485	59^{1} 59
480	61^{2} 61^{2}
475	$64_{}$ 64^{1}
470	$67_{}$ 67^{1}
465	70 69^{2}
460	72^{1} 72^{1}
455	$74_{}$ 74^{1}
450	77 77
445	79^{3} 79^{2}

EXPERIMENTAL DATA

BENZENE as the EXTRACTING MEDIUM

Wavelength of Maxium Absorption

Wavelength (mμ)	G(% Transmittance)
590	92^{1} 92
595	94^{1} 94
600	95 95
605	96^{2} 96^{2}
610	96 96
615	97^{2} 97
620	97 97^{3}
625	97 97^{2}
510	52 52^{8}
505	53^{1} 53^{1}
500	53^{2} 54
495	55^{3} 55^{3}

EXPERIMENTAL DATA

REFERENCE as the EXTRACTING MEDIUM

Transparency of Medium Absorption

Wavelength (mμ)	% (% Transmittance)
500	92
595	94
600	95
605	96
610	96
615	97
620	97
625	97
630	98
635	98
640	98
645	98

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

Having established that the source of the non-linearity of the benzene method is not inherent in the colored complex itself, it remained only to promote conditions to bring about the desired results. On close inspection of the data, it was obvious that benzene, as an extracting medium, was more efficient at the lower concentrations. It was thought that perhaps a more efficient extraction could be accomplished if the portion of benzene used was divided into two equal parts and a double extraction made. Thus an attempt was made to facilitate a greater recovery of the quinidine from the aqueous phase. Unfortunately, the results using the latter technique were identical with the results of a single extraction. Evidently, oneof two explanations was in order. First, either the single extraction removed all the quinidine or secondly, the amount of quinidine removed over and above that removed by a single extraction was not within the sensitivity of the apparatus and could not be detected. The problem was-- which of the two explanations was consistent with fact? On the answer to such a question rested the advisibility of continuing with benzene. If the first explanation alone were the real one, then essentially nothing could be done to improve the benzene method. No other solvent could be employed to bring

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

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about a more favorably distribution since the maximum extraction and are not considering the effect of benzene of promoting association and dissociation of the colored complex. Obviously, the solution was tied up with the distribution of quinidine between water and benzene. Accordingly, the volume of benzene used was cut down to 30 ml so that optimal extracting conditions would not be approached. A single extraction using 30 ml of benzene was performed and a double extraction using two 30 ml portions of benzene. The difference between the L values of the two procedures followed represented that quinidine not removed by the single 30 ml portion. The L determined by the double extraction was about 33% higher than that of the single extraction. Evidently, the distribution of quinidine between water and benzene is poor for extracting purposes. A rough calculation of the distribution coeficient would be as follows:

$$\frac{\frac{7}{30}}{\frac{3}{5}} = \text{Distribution Coeficient} = .4$$

That is, of a 10 microgram sample, only 7 micrograms was removed by the 30 ml of benzene. Even when the amount of benzene was increased to 47 ml only 8 out of 10 micrograms

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

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$$\frac{\frac{7}{30}}{\frac{23}{30}} = \text{Distribution Coefficient} = .4$$

That is, of a 10 microgram sample, only 7 micrograms was removed by the 30 ml of benzene. Even when the amount of benzene was increased to 47 ml only 8 out of 10 micrograms

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

were removed. So, as a temporary answer to the question posed above we may say that all the quinidine was not removed and that the additional amount removed by a second extraction could not be detected since it amounted to only 1.2 micrograms. The latter figure is easily calculated from the above equation.

Perhaps the reader has been aware of at least one important fallacy in the above logic. It was assumed throughout the discussion that the final amount of quinidine measured was 100% of the amount of quinidine extracted from the aqueous phase by benzene. That is, if 8 micrograms were detected it meant only 8 micrograms were extracted by benzene. Such is not necessarily so. It is entirely possible for the distribution of quinidine to be much more favorable then calculated. The low recovery of quinidine might be due to a loss of some quinidine after it has been extracted from the aqueous phase. The latter is quite comptabile with the data already reviewed. It has already been mentioned that the absorption of quinidine on glass surfaces is unusually large and that a highly polar substance like isoamyl alcochol has to be introduced to prevent the absorption. Experimental evidence has already been cited to show this. Thus, most of the difficulties encountered with the benzene method, might be

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attributed to the polarity of the solvent.

With this in mind, I directed my attention to the possibilities of employing a new solvent. The new medium would have to be as good a quinidine solvent as benzene and in addition, be more highly polar. Such criteria were met in the substance toluene, the mono-methyl derivative of benzene.

Other than being a more polar substance, toluene has other advantages over benzene as a solvent. Toluene is less toxic than benzene and what is more important, no emulsions are formed when it is shaken with an aqueous phase. In the preparation of a standard curve, as has already been described, extractions are made from aqueous phases. The little emulsion that forms between benzene and water is not particularly troublesome, but it means either an additional centrifugation has to be made or else, and this is preferable, the mixture must stand for at least twenty minutes until the boundary between the benzene and water is sharp enough to allow one to aspirate off the benzene. One might suppose that a centrifugation would be more suitable since it requires less time, but it is a good principle to avoid unnecessary handling of the solutions because of the minute amounts worked with. Toluene offers one more advantage over benzene which at first does not seem too important. The boiling point of toluene is

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TOLUENE as the SOLVENT

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111° while that of benzene is 80°. This effectively means that a greater recovery of the solvent can be made when toluene is employed. It was found that about 43 ml of benzene could be recovered at the end of a determination while about 45 ml of toluene could be recovered. The difference of 2 ml is attributed to a greater evaporation of benzene during the centrifugations. To effect the same per cent recovery of benzene as one gets with toluene, one would have to stopper the centrifuge tubes. While each advantage of toluene over benzene, in itself, does not seem too important, taken collectively, they aid in the fulfilment of the criteria previously stated for a suitable clinical method.

The reagents, apparatus and procedure followed using toluene were exactly the same as those described previously under the benzene procedure with one exception. The initial shaking time was increased from 15 minutes to 30 minutes to insure equilibrium.

Discussion of Results Obtained:

The standard curve was prepared exactly as described under the benzene procedure. The observed per cent transmission of each resulting acid solution was converted into the corresponding L value and this plotted against the concentration of quinidine. The relationship between L and concentration was

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

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EXPERIMENTAL PROCEDURE

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linear from 2.5 to 10 micrograms. The method is not sensitive enough to measure accurately any amount of quinidine below 2.5 micrograms. The portion of the curve above 10 micrograms was not investigated for two reasons. First, levels above 10 micrograms per ml are not compatible with life and secondly, if the occasion arose to measure greater amounts of quinidine, the samples could be diluted so that they would fall within the range described.

Stability of the Colored Complex and Standard Quinidine Solution:

The pink color resulting when the methyl orange-quinidine complex is extracted into the HCL layer is quite stable. The transmission of the solution may be measured as much as 24 hours after the color has been developed without any significant change, provided that the solution has been refrigerated all this time. The solution should be allowed to come to room temperature before its per cent transmission is read in the Coleman Photometer.

The quinidine sulfate standard prepared in .1N sulfuric acid is stable, if kept refrigerated, for about one month. It is advisable to discontinue the use of the standard after such a period and prepare a fresh one.

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

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Stability of the Colored Complex and Standard Quinidine Solution:

The pink color resulting when the methyl orange-quinidine complex is extracted into the HCl layer is quite stable. The transmission of the solution may be measured as much as 24 hours after the color has been developed without any significant change, provided that the solution has been refrigerated all this time. The solution should be allowed to come to room temperature before its per cent transmission is read in the Coleman Photometer.

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EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

The Effect of Varying Amounts of Methyl Orange:

As was previously stated, a saturated solution of methyl orange, was substituted for Brodie's solution to insure an excess of methyl orange. Since the water blanks do not have 100% transmission, it was thought that perhaps some methyl orange dissolved in the toluene and contributed the slight color of the water blanks. Thus, the amount of methyl orange might be a critical factor. Two distilled water blanks were run, adding to the first 1 ml of methyl orange and to the second 1.5 ml of methyl orange instead of the .5 ml normally used. The per cent transmission measured was exactly the same in both instances and was equal to the per cent transmission obtained when .5 ml of methyl orange was used. From this it may be concluded that the amount of methyl orange is not critical--provided there is enough to combine with all the quinidine present.

Reagent Blanks:

I chose not to follow Brodie's advice of using a reagent blank to set the Coleman at 100% transmission. In place of the reagent blank I used 1.5 ml 1N HCL. By employing such a device, I could eliminate the necessity of running reagent blanks concurrently with each quinidine determination. The per cent

TOLUENE as the SOLVENT

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EXPERIMENTAL PROCEDURE

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transmission of a number of reagent blanks, therefore, had to be determined and the average L of these obtained. By subtracting this average reagent blank from the value obtained with quinidine solutions, the color due to the methyl orange-quinidine complex could be arrived at. The reagent blanks were consistent and gave an average L value of .0064, less than that obtained with the ethylene chloride method.

Plasma Blanks:

The toluene procedure was tried on 1 ml of plasma diluted to 5 ml with water and on 5 ml of plasma alone, to which no quinidine had been added. The plasma was obtained from human blood. Coagulation of the blood was prevented either by adding 10 ml of a 5% sodium citrate solution to 50 ml of blood or else by adding 2 mg of potassium oxalate to each ml of blood. The oxalated and citrated plasma blanks were essentially the same, and the results obtained with 1 ml of plasma were identical with the results obtained with 5 ml of plasma.

As would be expected, the procedure as outlined previously, could not be applied per se to plasma and serum solutions. It became necessary to introduce a few modifications to deal with the precipitated proteins. When the toluene and alkaline plasma solutions are shaken, they do not

EXPERIMENTAL PROCEDURE

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EXPERIMENTAL PROCEDURE

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separate sharply enough to allow the toluene to be aspirated off without too great a loss. It was found that the precipitate must be allowed to settle and the mixture centrifuged before one can proceed to aspirate off the toluene. The procedure finally adopted for plasma and serum solutions will be discussed in length under the section of recoveries.

Serum Blanks:

Blanks were run on 1 ml of horse and human serum diluted to 5 ml and on 5 ml of serum to which no quinidine was added. The results in all cases were identical with each other and with the plasma blanks. Serum is easier to work with, since the fibrinogen has been removed. Thus, the precipitated proteins are less voluminous and easier handled.

Recoveries From Serum:

The procedure, employing toluene as the solvent, followed in performing the recoveries of quinidine from serum is as follows:

Add 1 ml of serum, 4 ml of water and 1 ml of 10% sodium hydroxide to 47 ml of toluene in a 50 ml glass-stoppered graduate cylinder and shake for 30 minutes by hand. Allow the precipitate to settle. Add 0.5 ml of isoamyl alcohol and mix with the toluene phase without disturbing the aqueous phase or precipitate. Transfer the

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

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mixture to a 100 ml centrifuge tube carefully and centrifuge for 10 minutes at 2500 R.P.M. Aspirate the toluene layer into a test tube and pour all of this into a 100 ml volumetric flask containing 0.5 ml of the saturated methyl orange solution. Shake for one-half hour on the shaker. Centrifuge the solution for 10 minutes at 2500 R.P.M. Aspirate off the toluene layer again and pour 43 ml of it into a 100 ml volumetric flask containing 2 ml of 1N HCL. Shake for one minute byhand vigorously, and for 20 minutes on the shaker. Centrifuge the solution for 10 minutes at 2500 R.P.M. Aspirate off the toluene layer. Insert a 2 ml pipette into the acid layer and transfer 1.5 ml of it to a microcoleman tube and read the per cent transmission of the solution, setting the instrument to read 100% transmission with 1.5 ml of 1N HCL, at a wave length of 515 mμ.

One has to be careful not to disturb the serum solution or protein precipitate when mixing the isoamyl alcohol with the toluene, as the solubility of quinidine in isoamyl alcohol is very great. If the mixture were shaken after the alcohol were added, the alcohol would serve to extract some quinidine from the serum solution and contribute it to the quinidine extracted by the toluene. The high solubility of quinidine

EXPERIMENTAL PROCEDURE

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was demonstrated by dissolving 100 mg of quinidine sulfate in water and another sample in isoamyl alcohol. Volume for volume, it took less alcohol to dissolve the quinidine sulfate. Because of this high solubility of quinidine sulfate in isoamyl alcohol, it was thought that the isoamyl alcohol might serve as the extracting medium in place of benzene or toluene. Unfortunately, when the highly polar isoamyl alchol is shaken with water emulsions result similiar to those incurred when ethylene chloride is shaken with water.

In performing the recoveries of quinidine, serum from patients was not used, but rather a stock solution of quinidine sulfate was prepared in serum. 5 ml of the standard solution used in determining the standard curve was diluted to 50 ml with horse serum yielding a final solution containing 10 micrograms of quinidine per ml of serum.

The method as stated, cannot measure any amount of quinidine below 2.5 micrograms. It was thought likely that 5 ml of serum could be used in those cases where the concentration of quinidine was below 2.5 micrograms per ml in an attempt to bring the total amount of quinidine within the range of sensitivity, but the recover of quinidine from 5 ml of serum is not good.

Most of the criteria for a successful clinical method for the quanitative determination of quinidine in serum have been

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

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Most of the criteria for a successful clinical method for the quantitative determination of quinidine in serum have been

EXPERIMENTAL PROCEDURE

TOLUENE as the SOLVENT

fulfilled. The method requires only 1 ml of serum; it is simple, quick and sensitive enough to measure any amount of quinidine above 2.5 micrograms. The method developed is in no sense of the word original. It simply represents an adaptation and extension of Brodie's work.

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EXPERIMENTAL DATA

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0.0	98^{2}	.0066
	98^{2}	.0066
	98^{2}	.0066
	98^{2}	.0066
	98^{2}	.0066
	98^{3}	.0055
2.5	91	.0398
	90	.0434
	91	.0414
5.0	84	.0757
	84	.0731
	85	.0706
7.5	79	.1024
	79_{1}	.1024
		.1010
10.0	73	.1352
	72	.1412
	75	.1235

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

Quinidine Standard Curve

Micrograms Quinidine/5ml	Corrected L (minus blank)	Corrected L / Concentration or K	Standard Error of Mean K SD_m
2.5	.0334	.01335	
	.0370	.01482	
	.0346	.01384	
5.0	.0693	.01383	
	.0667	.01335	
	.0642	.01262	
7.5	.0960	.01282	
	.0960	.01282	
	.0946	.01262	
10.0	.1288	.01288	
	.1348	.01348	
	.1171	.01171	
			.00022

Quinidine Standard Curve

Calculations

a. Sum of the individual K's	.15838
b. Mean K	.013198
c. Sum of K - Squared	.0250842244
d. $\sum \frac{\text{K-Squared}}{N}$ (N equals 12)	.0020903520
e. Sum - of K Squared	.0020969690
f. Sum of K Squared Minus $\frac{\text{K Squared}}{N}$ (Sum of the Deviations Squared-=D)	.0000066170
g. $\sum \frac{\text{D Squared}}{N-1}$	.0000006015
h. $\sum \frac{\text{D Squared}}{N(N-1)}$	.000000050125
i. $\sqrt{\sum \frac{\text{D Squared}}{N-1}}$ (Standard Deviations)	.00078
j. $\sqrt{\sum \frac{\text{D Squared}}{N(N-1)}}$ (Standard Error of Mean)	.00022

Quintiline Standard Curve

Calculations

a. Sum of the individual X's .15838

b. Mean X .013198

c. Sum of X - Squared .0258064224

d. $\frac{\text{X-Squared}}{N}$ (N equals 12) .0020903520

e. Sum - of X Squared .0020958520

f. Sum of X Squared Minus $\frac{\text{X Squared}}{N}$ .0000055170

(Sum of the Deviations Squared-=D)

g. $\frac{\text{D Squared}}{N-1}$ .0000000018

h. $\frac{\text{D Squared}}{N(N-1)}$ .0000000001258

i. $\frac{\sqrt{\text{D Squared}}}{N-1}$ (Standard Deviations) .00078

j. $\frac{\sqrt{\text{D Squared}}}{N(N-1)}$. (Standard Error of Mean=) .00022

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

A. Distilled Water Blanks:

ml of Water	G(% Transmittance)	L(2-Log G)
5	98^{2}	.0066
5	98^{2}	.0066
5	98^{3}	.0055
5	98^{2}	.0066
5	98^{2}	. 0066

B. Horse Serum Blanks:

ml of Horse Serum	G(% Transmittance)	L(2-Log G)
1	99^{1}	.0033
1	99^{1}	.0033
1	$99_{}$	.0044
1	99^{2}	.0022
5	99^{1}	.0033
5	99^{1}	.0033

C. Citrated Human Plasma Blanks:

ml of Plasma	G(% Transmittance)	L(2-Log G)
1	99^{2}	.0022
1	99^{2}	.0022
1	99^{1}	.0033
1	99^{2}	.0022

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

A. Distilled Water Blanks:

ml of Water	θ(% Transmittance)	1(2-log θ)
5	98_5	.0066
5	98_5	.0066
5	98_5	.0085
5	98_5	.0066
5	98_5	.0066

B. Horse Serum Blanks:

ml of Horse Serum	θ(% Transmittance)	1(2-log θ)
1	99_1	.0033
1	99_1	.0033
1	99_1	.0044
1	99_2	.0022
5	99_1	.0033
5	99_1	.0033

C. Diluted Human Plasma Blanks:

ml of Plasma	θ(% Transmittance)	1(2-log θ)
1	99_2	.0022
1	99_2	.0022
1	99_1	.0032
1	99_2	.0022

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

D. Oxalated Human Plasma Blanks:

ml of Plasma	G(% Transmittance)	L(2-Log G)
1	99^{1}	.0033
1	99^{1}	.0033
5	99^{1}	.0033
5	99^{1}	.0033

E. Human Serum Blanks

ml of Serum	G(% Transmittance)	L(2-Log G)
1	99^{1}_{1}	.0033
1	99^{1}	.0033
5	99^{1}_{1}	.0033
5	99^{1}	.0033

F. Effect of Methyl Orange on Distilled Water Blanks:

ml of Methyl Orange	G(% Transmittance)	L(2-Log G)
1	98^{2}	.0066
1.5	98^{3}	.0055

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

D. Oxalated Human Plasma Blanks:

ml of Plasma	σ (% Transmittance)	L(3-Log σ)
1	99_1^1	.0033
1	99_1	.0033
5	99_1^1	.0033
5	99_1	.0033

E. Human Serum Blanks

ml of Serum	σ (% Transmittance)	L(3-Log σ)
1	99_1^1	.0033
1	99_1	.0033
5	99_1^1	.0033
5	99_1	.0033

F. Effect of Methyl Orange on Distilled Water Blanks:

ml of Methyl Orange	σ (% Transmittance)	L(3-Log σ)
1	98_2	.0066
1.5	98_2	.0058

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

Recovery of Quinidine Added to Serum

ml of Serum	Quinidine (Micrograms)	G (% Transmittance)	L (2-Log G)
1	10	72[2]	.1397
1	10	72	.1427
1	10	73	.1367
1	10	74	.1308
1	5	86	.0655
1	5	85[1]	.0693
1	5	86[2]	.0630
1	5	89	.0505
1	5	85	.0706
1	5	88	.0555

ml of Serum	Corrected L	Am't Recovered	% Recovery
1	.1364	10.3	103
1	.1394	10.5	105
1	.1334	10.1	101
1	.1275	9.6	96

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

Recovery of Quinidine Added to Serum

ml of Serum	Quinidine (Micrograms)	% Transmittance	I (2-log %)
1	10	72	.1397
1	10	72	.1427
1	10	73	.1367
1	10	74	.1308
1	8	86	.0655
1	8	88	.0553
1	8	86	.0630
1	8	89	.0505
1	8	85	.0706
1	6	88	.0555

ml of Serum	Corrected I	Am't Recovered	% Recovery
1	.1384	10.3	103
1	.1394	10.8	108
1	.1334	10.1	101
1	.1275	9.6	96

EXPERIMENTAL DATA

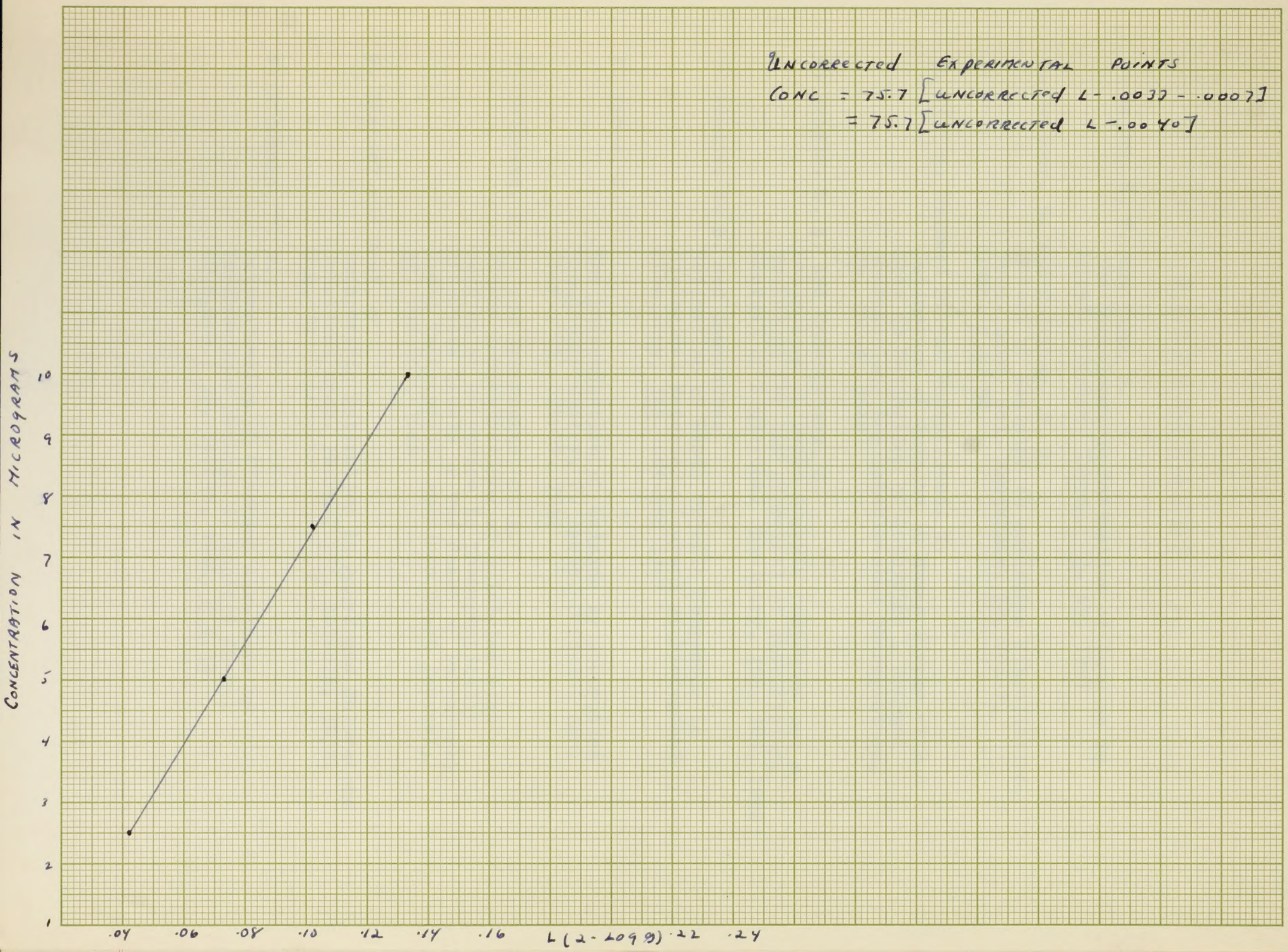
TOLUENE as the EXTRACTING MEDIUM

ml of Serum	Corrected L	Am't Recovered	% Recovery
1	.0622	4.7	94
1	.0660	4.9	98
1	.0597	4.5	90
1	.0472	3.6	72
1	.0673	5.0	100
5	.0522	3.8	76

EXPERIMENTAL DATA

TOLUENE as the EXTRACTING MEDIUM

ml of Serum	Corrected I	Mg't Recovered	% Recovery
1	.0822	4.7	94
1	.0820	4.9	98
1	.0827	4.5	90
1	.0872	3.6	72
1	.0873	5.0	100
5	.0822	3.8	76


UNCORRECTED EXPERIMENTAL POINTS
CONC = 75.7 [UNCORRECTED L - .0033 - .0007]
= 75.7 [UNCORRECTED L - .0040]
CONCENTRATION IN MICROGRAMS
10
9
8
7
6
5
4
3
2
1
.04
.06
.08
.10
.12
.14
.16
L (2 - LOG B)
.22
.24

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